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CORRELATION BETWEEN MICROBIAL POPULATIONS AND SAP YIELDS FROM MAPLE TREES [a, b, c]

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An early decline and/or stoppage of sap flow from maple tree tapholes frequently occurs during the sap season. This has been commonly attributed to the drying effect of the air or wind on the surfaces of the taphole *(1, 2, 4)*. However, limited studies by Naghski and Willits *(5)* indicated that premature stoppage of sap flow was closely related to an increase in numbers of organisms within the taphole and not to any physical effect. They also proposed that high populations of microorganisms in the taphole would have an adverse effect on the quality of finished syrup.

Since the latter study was on too small a scale to bear statistical analysis, the first experiments in this work were designed to test their findings statistically. The second phase of this work was concerned with the isolation and identification of the various microorganisms occurring in the sap, and is reported elsewhere *(6)*. The next step was to determine what specific microorganisms found in the sap would result in a decline or stoppage of sap flow. Finally, methods of controlling or preventing microbial infection of the taphole were considered in an attempt to find a practical means of controlling microbial activity; and, thus, increase the quality and quantity of sap produced.

EXPERIMENTAL

The experimental work in this investigation was conducted during a three-year period including the 1955, 1956 and 1957 sap production seasons. A maple sugar bush located on the campus at East Lansing, containing previously untapped maple trees, was used for the tapping and sampling studies. Two techniques of tapping were employed. The commercial or regular technique involved using commercial type spiles in 7/16-inch tapholes. The "aseptic" technique was the same as described by Naghski and Willits *(5)* except that sterile 5-gallon cans were used as collection containers instead of the glass carboys. Although all precautions possible were used to exclude microbial contamination, not all of the "aseptic" type tappings were originally in a state of microbial asepsis and none remained in this condition until the very end of the sap seasons. Therefore, "aseptic" as used in this study, refers to this specific tapping procedure and not to the usual definition of this term.

Samples of sap were taken at intervals throughout the 3 seasons, usually during the major sap runs of each season. All samples were taken with sterile equipment, either directly from the running tapholes or from the sap collection containers. The sap produced by the various tappings was measured by weighing to the nearest tenth of a pound each day on which a sap run occurred.

Populations of bacteria, yeasts and molds were determined in all samples using standard plating and counting techniques. Nutrient agar was used for the enumeration of the

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bacteria; and dextrose agar containing 0.1% yeast extract and acidified with 2 ml. of 5% tartaric acid per 100 ml. was used for yeast and mold counts. All plates were incubated at room temperature for 3 to 5 days.

RESULTS

Comparison of "Aseptic" and regular tappings. To investigate the relationship existing between premature decline in sap production and microbial activity within the tapholes, "aseptic" and regular tappings on the same trees were compared with respect to microbial populations in the sap and yields of sap. The effect of various dates of tapping on microbial activity in the tappings and on sap yields was also studied during the 1955 season.

Ten trees were tapped on each of 5 dates spaced at approximately 15-day intervals beginning on January 10, 1955. The dates were chosen so that the first 3 dates would fall before the normal tapping period for the East Lansing area, the fourth date during this period, and the last date after the normal tapping time. Each tree was tapped with an "aseptic" and a regular tapping 6 inches apart on the same quadrant of the tree according to compass position. Microbial populations in the sap and sap yields were determined throughout the season.

The "aseptic" tapping procedure was quite successful in delaying the appearance of high populations of microorganisms in tapholes bored early in the season. Thus, the average microbial populations in the sap from the regular tappings made January 10, January 25 and February 10 had attained high levels prior to any appreciable numbers appearing in the sap from the "aseptic" tappings (Fig. 1). However, the difference between the 2

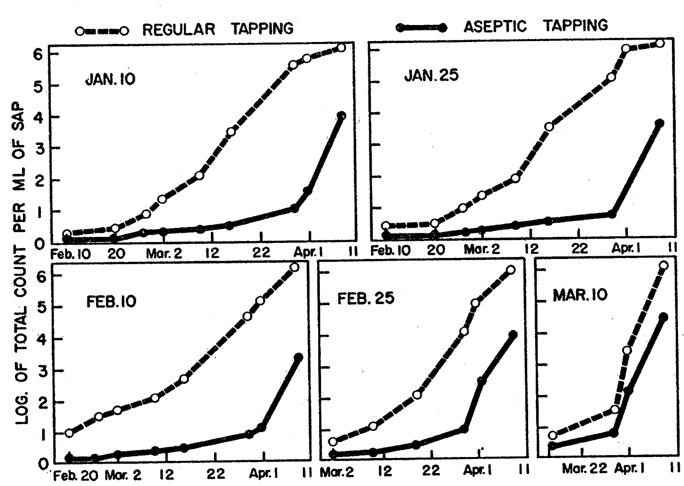

Figure 1. Populations of microorganisms in sap from regular and "aseptic" tappings.

types of tappings was much less in the February 25 tappings; and was not significant in the March 10 tappings. This demonstrates that the so-called "aseptic" tappings were not sterile; and that the relatively warm weather occurring during the last part of the sap season encouraged rapid growth of microorganisms in both types of tappings.

High populations of microorganisms early in the season in the sap from tapholes were accompanied by low average yields. This effect is illustrated by the percent increase in yield resulting from "aseptic" tapping procedures (Fig. 2). These percentages were calculated as follows:

$$\% = \frac{\text{lbs sap from "aseptic"} - \text{lbs sap from regular}}{\text{lbs sap from regular}} \times 100$$

The increased yield obtained by "aseptic" tapping is quite evident for the first 3 tapping dates; but was insignificant for the last two. Analysis of variance of the yield data demon-

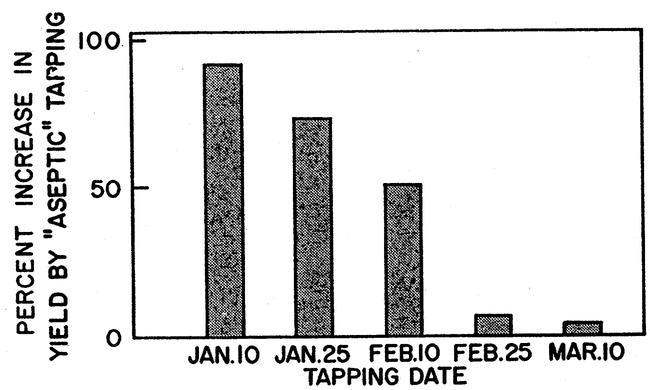

Figure 2. Increased sap yield resulting from "aseptic" tapping.

strated a highly significant difference (1% level) between the 2 tapping techniques for the January 10 tapping date; and significant differences (5% level) for the January 25 and February 10 tappings.

To obtain a statistical evaluation of the correlation between increased microbial activity within the taphole and the decrease in yield resulting from this activity, it was first necessary to establish a microbial population level in the sap considered to represent a condition of incipient decline in yield from the taphole. Examination of microbial population data indicated that 10^4 organisms per ml. of sap could be considered to represent a high level of microbial activity in the taphole which would eventually result in stoppage of sap flow. A correlation analysis was computed using all data for "aseptic" and regular tappings from the 1955 and 1956 seasons. The percent increased yield produced by the "aseptic" (A) as compared with the regular (R) tapping was correlated with the days difference between dates of attainment of 10^4 organisms per ml. of sap in the "aseptic" and regular tappings. Figure 3 shows the predicting line and its equation, together with the standard error of estimate of this predicting line. A correlation coefficient of 0.66 was calculated. This correlation was determined to be significant at the 1% level.

Inoculation Studies: To further test the effect of microorganisms on sap yields from maple trees and to ascertain what particular organisms were responsible for the stoppage

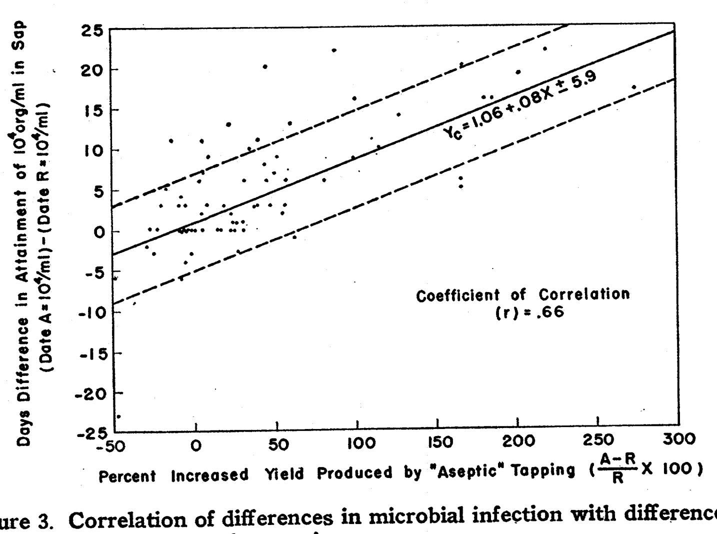

Figure 3. Correlation of differences in microbial infection with differences in sap yield from "aseptic" and regular tappings.

of sap flow, an inoculated tapping was included in the second season's work. Therefore, each tree had 3 tapholes spaced 6 inches apart: one regular, one "aseptic," and the third tapped by the "aseptic" procedure but inoculated with 2 ml. of a suspension of one or more microorganisms. The cultures used were those found to be most prevalent in sap from tapholes *(6)*. Four trees were inoculated with *Pseudomonas geniculata*, and 2 trees with each of the following: *Pseudomonas putrefaciens, Flavobacterium solare, Achromobacter sp., Micrococcus varians, Candida sp., Rhodotorula glutinis, Torulopsis aeria, Cryptococcus laurentii*, a culture of a large group similar to *Trichosporon pullulans, Penicillium sp.*, a mixture of the five yeast cultures, a mixture of the five bacteria cultures, and a mixture of all bacteria and yeast cultures. Of the total 30 trees tapped, 15 were tapped February 7, and 15 on February 29.

Microbiological studies of sap from these tappings were made throughout the season. In general, the sap from tapholes inoculated with bacteria had initial populations of about 10^4 per ml. and final populations of about 10^7; and that from tapholes inoculated with yeasts had initial levels of about 10^3 and final levels of about 5×10^5. There was a fairly constant increase in the number of these organisms throughout the season. The sap from the 2 tapholes inoculated with mold did not develop high numbers of molds. However, reamings from these tapholes made at the close of the season revealed that they were heavily infested with mold hyphae.

No high microbial numbers were noted in the sap from either the regular or "aseptic" tappings until very late in the season. This probably resulted from the uniformly cold weather during February and most of March, 1956.

Effect of taphole inoculations on sap yield is shown in Table 1. The inoculated tap-

TABLE 1

Sap yields from "aseptic", regular, and inoculated tappings. (1956 season)

Date of tapping	Average yield in pounds of sap		
	"Aseptic"	Regular	Inoculated
Feb. 7	152.4	126.7	57.3
Feb. 29	149.8	121.1	77.9
Average	151.1	123.9	67.6 **

** Significant at the 1% level.

pings of February 7 yielded only about one-third of the total amount of sap produced by the "aseptic" tappings, whereas the inoculated tappings of February 29 produced about one-half of the amount of sap from the "aseptic" tapholes. Analysis of variance calculations with these data showed a highly significant difference between yields of regular and inoculated tappings and between yields of "aseptic" and inoculated tappings. There was about a 22% greater yield from the "aseptic" than from the regular tapholes, but this did not prove to be significant. This might be expected due to the relatively low numbers of microorganisms observed in the regular tappings of this season.

Of particular interest was the fact that all microorganisms tested reduced sap flow. Every inoculated tapping had either completely stopped producing sap or had appreciably diminished in flow at least 2 weeks before the uninoculated tappings. This appears to be a very non-specific effect insofar as the type of microorganism is concerned.

A correlation coefficient was obtained from data similar to those shown in Figure 1 for the "aseptic" and inoculated tappings. The equation of the predicting line was found to be $Yc = 28.92 + .03 \times \pm 6.7$. The coefficient of correlation (r) was 0.58, which is significant at the 1% level.

Treatments of tapholes to prevent microbial growth or contamination. The "aseptic" tapping technique would be impractical under commercial conditions. Therefore, studies were conducted with microbial inhibitors and sanitizers added to the tapholes. Also, an attempt was made to reduce the amount of microbial contamination of the taphole by the use of plastic tubing running from the spiles to the collection containers.

Preliminary studies using aureomycin and hypochlorite solutions as rinses and inserting sorbic acid pellets in tapholes proved to be unsuccessful in controlling microbial growth in the tapholes. This might have been expected since the sorbic acid is relatively ineffective at the pH of sap, the aureomycin has no effect on the yeasts and molds, and the hypochlorite is destroyed very rapidly in the presence of organic material.

More drastic treatments for controlling the microorganisms were then resorted to. These were: (a) paraformaldehyde (trioxymethylene) in agar pellets, (b) paraformaldehyde in plaster of paris pellets, (c) mercuric iodide in plaster of paris pellets, (d) "Sterilite"[f] powder added directly to the taphole, and (e) "O-Silver" aqueous[g] applied in suspension to cotton stuffed in the tapholes. A group of 14 trees was tapped for each of these treatments with each tree having a treated and an untreated taphole. Both the control and treated tappings in 7 trees of each group were inoculated with 2 ml. of a broth culture of *Ps. geniculata*.

Microbiological studies showed that all treatments, except the "O-Silver" suspension, did an excellent job of preventing growth of microorganisms in the tapholes. The type of control obtained is illustrated by the data for the trees treated with paraformaldehyde-agar pellets in Figure 4. While little or no control was evident with the "O-Silver" treatment (Fig. 4), this may well have been due to the method of treatment.

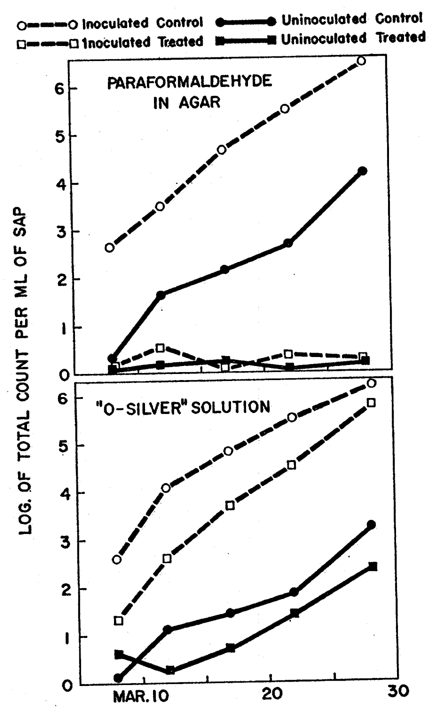

Figure 4. Effect of paraformaldehyde and "O-Silver" on the development of microorganisms in maple tree tapholes.

An additional experiment was run testing the effect of using Tygon plastic tubing to conduct the sap from the spile to the collection container. No inoculations were used in these experiments. Tubing which had been treated with silicone was used on the treated tapping on seven of the trees in this experiment and regular tubing on the other seven. The data indicated some reduction in number of microorganisms in the sap from the tapholes with plastic tubing. However, the control was far from complete and considerable variation was noted among replications.

[f] The "Sterilite" powder, a preparation of colloidal carbon coated with colloidal silver, was supplied by the Shellmar-Betner Flexible Packaging Division of Continental Can Co., Inc., Mount Vernon, Ohio.

[g] The "O-Silver" aqueous, a germicidal suspension of oligodynamic silver, was supplied by The Chloromine Co., 54 West 16th Street, New York 11, New York.

Yield data were collected for all the treated tappings except for those treated with mercuric iodide. The effect of the two paraformaldehyde treatments and the "Sterilite" powder treatment on yield of sap was quite pronounced (Table 2). An analysis of variance of these data showed that the yields from these three treatments were significantly higher than the corresponding controls. In fact, the average yield from tapholes treated with the paraformaldehyde in agar was approximately four times that from the corresponding controls. The "O-Silver" and plastic tubing treatments did not result in significantly greater yields.

TABLE 2

Effect of various treatments on average yields of maple sap

Treatment [1]	Inoculation	Average yields in pounds	
		Treated	Untreated
Paraformaldehyde in agar	Uninoculated [2]	319.8	105.1
	Inoculated	349.4	64.0
	Average	334.6	84.5 **
Paraformaldehyde in plaster	Uninoculated [2]	271.4	191.0
	Inoculated	305.6	74.8
	Average	288.5	132.9 *
Sterilite powder	Uninoculated [2]	243.3	202.7
	Inoculated	260.9	116.1
	Average	252.1	159.4 **
O-Silver solution	Uninoculated [2]	138.7	172.4
	Inoculated	99.4	87.4
	Average	118.6	129.9
Plastic tubing	Silicone coated [3]	191.1	154.0
	Uncoated	257.7	190.9
	Average	224.4	172.5

[1] Fourteen trees were tapped per treatment with a treated and a control tapping in each tree.
[2] Both tapholes were inoculated on 7 trees and uninoculated on the other seven.
[3] The plastic tubing used on the treated tappings was silicone coated on 7 trees and not coated on the other seven.
** Significant at the 1% level.
* Significant at the 5% level.

DISCUSSION

Growth of microorganisms (bacteria, yeasts, or molds) in maple tree tapholes results in a decline in flow rate and, frequently, complete stoppage of sap flow. These data confirm the preliminary observations of Naghski and Willits *(5)*, and refute the popular belief that the physical drying by air and wind causes premature stoppage of maple sap flows. The extent of this effect on sap yield will vary greatly from tree to tree and with the season. The tree variation is probably the result of differences in initial contamination and the rate of infection of the tissues around the taphole. Differences in average temperatures of sap seasons would undoubtedly influence the rate of development of microorganisms within the taphole. Such a difference was indicated in this study by comparison of the data for the 1955 season with that of 1956. The season was quite cold in 1956 compared to 1955, and microbial development in the regular tapholes of 1956 did not occur nearly as early as in 1955. In correlation with this, the average yield differences between the two types of tappings made early in February was significant in 1955 but not in 1956. However, when the initial populations of microorganisms were high, as a result of inoculation, yields were greatly reduced even in a relatively cold season. The amount of precipitation and wind occurring during the season may have an influence on the amount of natural contamination of tapholes; and, thus, indirectly influence yields. Still another variable, is the time of exposure of the taphole to natural contamination and to growth of microorganisms. Yields from trees tapped early in the season are much more likely to be affected by microbial growth than trees tapped at later dates.

The actual mechanism by which microorganisms stop the flow of sap from maple tree tapholes is not known. However, the fact that so many unrelated types and species of microorganisms will result in stoppage indicates that the effect may be a purely physical one. Histological studies of the tissues around infected tapholes conducted in conjunction with this work showed bacteria and yeast cells, and fungal hyphae in vessels and other types of cells *(3)*. Gummy substances containing numerous bacteria were frequently found plugging the open lumens and vessel elements of the tissue.

The control of microbial development in tapholes is a difficult problem. The tapholes are quite subject to initial contamination from the bark and surface tissue and to subsequent contamination through the spile and around the edges of the spile. The most promising method of control is the use of some good antimicrobial agent in a form that could be inserted into the taphole when it is bored. This would have to be incorporated in a pellet with a slow disintegration rate so there would be a residual in the taphole throughout the season. Also, this agent should either be volatilized or destroyed during the concentration of the sap to syrup. Paraformaldehyde incorporated into a pellet does a fine job of microbial control, but there is insufficient evidence available at this time with respect to its elimination from the sap during concentration. This is undergoing further investigation.

SUMMARY

A comparison has been made of microbial populations and sap yields from regular and experimentally inoculated tapholes in maple trees with tappings in which the development of microorganisms was partially or completely controlled by the use of special tapping procedures or chemical additives. This study was conducted over a three-year period, and was of sufficient scope to permit statistical analysis. A high degree of correlation was demonstrated between the occurrence of high populations of bacteria early in the sap season and low yields of sap. Therefore, it is concluded that microorganisms are the primary cause of premature stoppage of sap flow.

Inoculation studies with a number of species of bacteria and yeasts and one mold culture demonstrated that any organism capable of growing in the environment would result in stoppage of sap flow. Therefore, it is indicated that the organisms probably stop sap flow by physical blocking of the vessels. The amount of initial contamination and the average temperature affect the rate of development of the microflora; and, thus, influence the time at which a drying up of the taphole occurs.

Use of a pellet containing paraformaldehyde inserted into the taphole was the most promising of a number of methods tested for controlling microorganisms. However, insufficient data are available on the elimination of the formaldehyde from the sap during concentration to syrup to make any conclusions as to its usefulness at this time.

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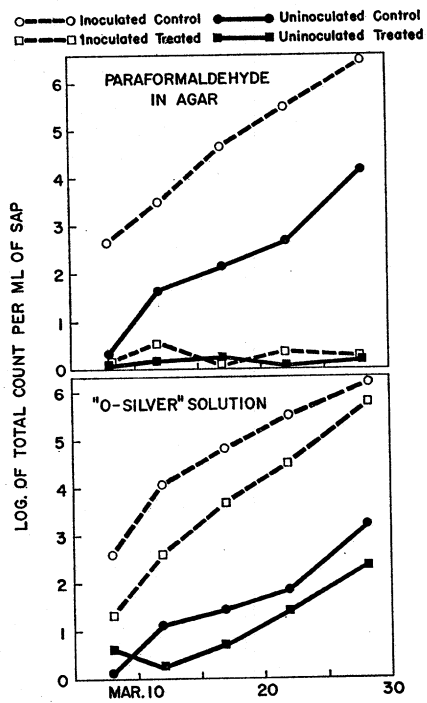

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